In Situ Genetic Correction of the Sickle Cell Anemia Mutation in Human Induced Pluripotent Stem Cells Using Engineered Zinc Finger Nucleases.

Journal: Stem Cells

Publication Year: 2011

Authors: V Sebastiano, M L Maeder, J F Angstman, B Haddad, C Khayter, D T Yeo, M J Goodwin, J S Hawkins, C L Ramirez, L F Batista, S E Artandi, M Wernig, K J Joung

PubMed link: 21898685

Funding Grants: Stanford CIRM Training Program

Public Summary:
The promise of induced pluripotent stem cells (iPSCs) in regenerative medicine is given by the fact that, since they can be derived from any patient, their use for clinical applications overcomes the problem of immune rejection. However the big challenge in the field is the development of reliable tools for correcting the genetic mutations that are causative of the development of the patients' diseases. Recently, engineered zinc finger nucleases (ZFNs) have been shown to be invaluable tools to manipulate the genome of iPSCs, raising the prospect of employing this technology to correct disease-causing mutations. Here we describe for the first time the generation of iPSCs lines from sickle cell anemia patients and in situ correction of the disease-causing mutation using three ZFN pairs. The strategy we adopted has several advantages: I) By using "molecular scissors" (ZFNs) we can correct a precise region of the genome without interfering with any other sequence; II) Since we corrected the endogenous sequence of the gene in its right genomic context, the full functionality of the gene is guaranteed. The gene-corrected cells that we generated retained full characteristics of normal and functional iPSCs even after all the genetic manipulations. Our approach delineates a roadmap for using ZFNs to achieve efficient correction of monogenic disease mutations in patient-derived iPS cells and by avoiding the integration of any exogenous DNA. Our results provide an important proof of principle that ZFNs can be used to produce gene-corrected human iPSCs cells that could be used for therapeutic applications.

Scientific Abstract:
The combination of induced pluripotent stem (iPS) cell technology and targeted gene modification by homologous recombination (HR) represents a promising new approach to generate genetically corrected, patient-derived cells that could be used for autologous transplantation therapies. This strategy has several potential advantages over conventional gene therapy including eliminating the need for immunosuppression, avoiding the risk of insertional mutagenesis by therapeutic vectors, and maintaining expression of the corrected gene by endogenous control elements rather than a constitutive promoter. However, gene targeting in human pluripotent cells has remained challenging and inefficient. Recently, engineered zinc finger nucleases (ZFNs) have been shown to substantially increase HR frequencies in human iPS cells, raising the prospect of employing this technology to correct disease-causing mutations. Here we describe the generation of iPS cell lines from sickle cell anemia patients and in situ correction of the disease-causing mutation using three ZFN pairs made by the publicly available Oligomerized Pool Engineering (OPEN) method. Gene-corrected cells retained full pluripotency and a normal karyotype following removal of reprogramming factor and drug-resistance genes. By testing various conditions, we also demonstrated that HR events in human iPS cells can occur as far as 82 bps from a ZFN-induced break. Our approach delineates a roadmap for using ZFNs made by an open-source method to achieve efficient, transgene-free correction of monogenic disease mutations in patient-derived iPS cells. Our results provide an important proof of principle that ZFNs can be used to produce gene-corrected human iPS cells that could be used for therapeutic applications.